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Isolation by FACS sorting for qRT-PCR of Drosophila larvae class IV da neurons

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An abbreviated version of this protocol was published in eLIFE in Mar 2022

Steroid hormone signaling activates thermal nociception during *Drosophila* peripheral nervous system development

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Detailed protocol

1. Prepare 30 larvae for each condition
2. Place larvae in microfuge tube with 1x PBS
3. Vortex 3x at maximum setting 1sec each. Discard supernatant. Add PBS. (repeat 3-4x)
4. Briefly wash with 1ml of 70% ethanol and discard supernatant.
5. Wash with 1ml PBS. (repeat 2x)
6. Wash with 500ml RNase-AWAY and discard the supernatant.
7. Wash with 1ml PBS. (repeat 3x, until suds/bubbles are gone)
8. Dissect larvae in ice cold PBS on ice
9. Invert larval body wall and remove the CNS, imaginal discs, gut, and fatbody
10. Add carcasses into tube with 500ul PBS on ice
11. Add 1.5 ul Liberase (1x Liberase TM (Roche, LIBTM-RO))
12. Vortex
13. Incubate for 5min on heated 25oC agitator at 1,000 rpm
14. triturate 10x with glass pipette* [Repeat steps 13 and 14 three times]
15. Strain through 40um cell strainer (Fisher Scientific)
16. Rinse and strain with 1000ul Schneider's to bring total volume to 1.5ml and inactivate Liberase
17. Add 1ul EthD-1 (ethidium homodimer-1(ThermoFisher, L3224))
18. Keep on ice until sorting. Before sorting prepare:
    a. 1.5 ml tubes with 20ul extraction buffer (Thermofisher, KIT0204)
    b. Box of dry ice
19. Isolate cells by fluorescence-activated cell sorting (FACS) with an Aria II (Becton Dickinson). Sort GFP+ nonautofluorescent RFP- events into lysis buffer.
20. After sorting, flash freeze on dry ice and store samples at -70oC
21. To begin RNA isolation, thaw samples on ice
22. Centrifuge at 800g for 1min
23. Incubate for 30min @ 42oC
24. Using PicoPure RNA Isolation Kit (Thermofisher, KIT0204), follow manufacture instructions for RNA isolation starting on page 14 Version D.
    a. Adjust Ethanol extraction volume to 20ul
    b. Use elution volume of 13ul
25. Synthesize cDNA following manufacture instructions for Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (ThermoFisher, 4368813)
26. Preform qRT-PCR using SYBR green (ThermoFisher A25742) with a QuantStudio7 (ThermoFisher). Calculate relative expression using the detla-delta Ct method with the housekeeping gene eEF1α2.

*Note: Narrow glass pipette tip in flame to match carcasses size in order to create mechanical strain during trituration.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Jaszczak, J. S., Devault, L. , Jan, L. Y. and Jan, Y. N.(2022). Isolation by FACS sorting for qRT-PCR of Drosophila larvae class IV da neurons. Bio-protocol Preprint. bio-protocol.org/prep1897.
2. Jaszczak, J. S., DeVault, L., Jan, L. Y. and Jan, Y. N.(2022). Steroid hormone signaling activates thermal nociception during *Drosophila* peripheral nervous system development. eLIFE. DOI: 10.7554/eLife.76464